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Sent: Saturday, March 01, 2003 2:46 PM
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Gregg et al. American Journal of Human Genetics, October 1997, Vol. 61, No. 4. SUPPL., ppA235, ISSN 0002-9297

Gregg et al. American Journal of Human Genetics, October 1997, Vol. 61, No. 4. SUPPL., 1363, ISSN 0002-9297
NOTE**** The references requested above are the same but two different page numbers were listed on STN.

Watts et al. American Journal of Human Genetics, October 2002, Vol. 71, No. 4 pages 791-800.]

Hacia et al. American Journal of Human Genetics, October 2000, Vol. 67, No. 4, Supplement 2, pp 66.

Wang et al, Oncogene, March 16, 2000, Vol. 19, No. 12, pages 1519-1528

thank You

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A deletion encompassing *Zic3* in Bent tail, a mouse model for X-linked neural tube defects. *B. Franke[1], R. Klootwijk[1], C.E.E.M. van der Zee[2], R.T. de Boer[3], H.W. van Straaten[4], W. Wilms[5], F.A. Hol[1], E.C.M. Mariman[1].* 1) Department of Human Genetics; 2) Department of Cellbiology; 3) Department of Anatomy, UMC Nijmegen, Nijmegen; 4) Department of Anatomy and Embryology, University of Maastricht, Maastricht; 5) Central Animal Laboratory, UMC Nijmegen, Nijmegen, The Netherlands.

Neural tube defects (NTD) are congenital malformations with an incidence of 1-2 per 1000 births. In NTD the neural tube fails to fuse completely during embryonal development leading e.g. to anencephaly or spina bifida. NTD is a multifactorial disease, being caused by the combined effect of environmental and genetic factors. To date over 60 mouse models have been described that enable the study of the genetic factors involved in NTD. Bent tail (Bn) is a mouse model for X-linked NTD. Bn mice are characterized by a kinked tail. In Bn embryos we have observed abnormalities including exencephaly, rotation defects, omphalocele and occasionally orofacial schisis. The exencephaly is consistently caused by a fusion defect of the neural tube in part of the midbrain and the hindbrain region. *Bn* maps to the proximal part of the X chromosome. In the Juli issue of *Human Molecular Genetics* we report the gene defect of Bn, a deletion encompassing the *Zic3* gene. *Zic3* is a homolog of the *Drosophila* segmentation gene *odd-paired* encoding a zinc finger transcription factor expressed in murine neuroectoderm during neurulation. Data on *Zic3* expression and function suggest that deletion of *Zic3* is the main cause of the Bent tail phenotype in the mouse. Recently, endclone analysis of YACs in the region of *Zic3* has shown that the Bn deletion spans less than 890 kb. Currently we are further characterizing the deleted area and we will show data on at least one additional gene that is deleted in Bn. In man mutations in *ZIC3* have already been shown to lead to situs abnormalities, occasionally associated with NTD. To assess the relevance of *ZIC3* as a risk factor for human NTD, mutation studies have been performed in a panel of sporadic as well as familial cases of NTD including a large Icelandic pedigree.

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The X-linked Mouse Mutation Bent Tail Is Associated with a Deletion of the Zic3 Locus. *T.L. Carrel[1], S.M. Purandare[2], W. Harrison[3], F. Elder[3], T. Fox[4], B. Casey[2], G.E. Herman[1].* 1) Department of Pediatrics, The Ohio State University, Columbus, OH; 2) Department of Pathology, Baylor College of Medicine, Houston, TX; 3) Department of Pathology, University of Texas Health Science Center, Houton, TX; 4) Department of Pathology, The Ohio State University, Columbus, OH.

The frequency of neural tube defects is second only to congenital heart disease in regard to congential malformations, with an estimated incidence of between 1-9/1000 total births. Several mouse mutants exist that are presumed models of human neural tube defects. Bent tail (*Bn*) is a spontaneous, semi-dominant, mutation on the mouse X chromosome that produces tail deformities and occassionally open neural tube defects. These defects are observed in homozygous females and hemizygous males as short, kinked tails. Heterozygous females show variable expression due to X inactivation. A backcross between inbred *Bn* and C3H mice was established, and PCR-based polymorphic microsatellites have been utilized to map the *Bn* locus as a prelude to isolation of the gene. Analysis of 292 normal male and affected male and female progeny places the *Bn* mutation in a 2.4 cM region between *DXmit166* and *DXmit140*. Refined genetic and physical mapping of the *Bn* critical region demonstrated that the mutation was associated with a <170 kb submicroscopic deletion that includes the anonymous microsatellite marker *DXMit208* as well as the the entire *Zic3* locus. In addition, no expression of *Zic3* can be detected by RT-PCR in affected *Bn* males. Human mutations in *ZIC3* are associated with left-right axis malformations. Similar anomalies were found in *Bn* males and females, in both the abdominal and thoracic cavities. The presence of anal and spinal abnormalities in some of the *ZIC3* mutant human patients and the deletion of *Zic3* in *Bn* mice support a key role for this gene in neural tube development and closure.

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Genotype-phenotype correlation in malformations caused by mutations in the p63 gene. *P. Ianakiev[1], M.W. Kilpatrick[1], I. Toudjarska[1], D. Basel[2], P. Beighton[2], P. Tsipouras[1].* 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Human Genetics, University of Cape Town, Observatory, South Africa.

Gene targeting studies have demonstrated that p63, a homologue of the cell-cycle regulator TP53, plays a critical role the formation and differentiation of the apical epidermal ridge (AER), a stratified epithelium essential for limb development. Mice lacking p63 have striking developmental defects including limb truncations, abnormal skin and absence of hair follicles, teeth and mammary glands. Split Hand-Split Foot (SHFM)is a limb malformation involving the central rays of the autopod presenting with median clefts of the hands and feet, absence of fingers and toes and syndactyly. There is phenotypic overlap with the EEC (ectrodactyly, ectodermal dysplasia, facial cleft) syndrome, in which distal limb malformations sometimes occur as part of the phenotypic spectrum. We have identified two missense mutations, 724AG predicting an amino-acid substitution K194E and 982TC predicting an amino-acid substitution R280C, in exons 5 and 7 respectively of the p63 gene in two families with SHFM and two mutations in families with EEC syndrome; 279RH and 304RQ. All four mutations fall within the DNA binding domain of the p63 molecule. The possible consequences of the mutations were assessed by building a model of the DNA binding domain of the p53 protein. The two amino-acids mutated in the SHFM families appear primarily to be involved in maintaining the overall structure of the domain, whereas the p63 mutations responsible for EEC syndrome reside in amino acid residues that directly interact with the DNA. This raises the possibility that the phenotypic distinction between SHFM and EEC syndrome arise as a consequence of this difference. The Adams-Oliver syndrome (AOS),in which variable distal limb reduction abnormalities occur in conjunction with defects of the calvarium and scalp, also overlaps phenotypically with SHFM and EEC. In addition to expanding our analysis of the p63 gene in SHFM and EEC families, we are investigating its putative role in AOS. In this way we hope to further understand the relationship between different mutations in the p63 gene and the phenotypes they produce.

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TGFβ mediates impaired alveologenesis in fibrillin-1 deficient mice. *E.R. Neptune[1], P.A. Frischmeyer[1], D.E. Arking[1], T.E. Bunton[1], L. Myers[1], F. Ramirez[2], H.C. Dietz[1,3].* 1) Institute of Genetic Medicine, Johns Hopkins SOM, Baltimore, MD; 2) Mt. Sinai SOM, New York, NY; 3) HHMI.

Marfan syndrome (MFS) is a heritable disorder of connective tissue caused by mutations in fibrillin-1, a constituent of extracellular microfibrils. Although the major manifestations are in the cardiovascular and skeletal systems, ~11-15% of pts manifest lung disease characterized by emphysematous changes which predispose to spontaneous pneumothorax. Current pathogenetic models invoke a destructive process that is initiated by physiologic stress acting upon a tissue that lacks structural integrity. We have now had the opportunity to test this hypothesis in fibrillin-1 deficient mice that are homozygous for targeted Fbn1 alleles and recapitulate the vascular phenotype of MFS. A striking deficiency in distal septation of alveoli was evident at birth and maintained until the time of vascular death (~10 PD). An intermediate phenotype was observed in the heterozygous animals. Elastin deposition was preserved and ultrastructural analysis revealed normal distribution of elastin at the tip of primordial septae. In that excessive TGFβ1 signaling was previously associated with alteration of distal branching morphogenesis and fibrillin-1 contains domains with homology to latency-inducing TGFβ binding proteins, we hypothesized a role for this family of cytokines in the lung phenotype of MFS. An antibody specific for active TGFβ1 was used to demonstrate a dramatic increase in immunoreactivity within the lungs of fibrillin-1 deficient mice. We utilized a novel transgenic reporter allele, comprised of tandem TGFbeta-responsive promoter elements upstream of the gene encoding green fluorescent protein, to document a 4-fold and 25-fold increase in TGFβ signaling in vivo in heterozygous and homozygous Fbn1-targeted mice, respectively. Intraperitoneal injection of TGFβ neutralizing antibody at birth rescued lung branching in the heterozygote animals. These data document a role for fibrillin-1 in modulating local active concentrations of TGFβ and demonstrate that perturbation of this regulation underlies primary failure of lung branching morphogenesis and perhaps other manifestations of MFS.

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Mice doubly mutant in the Fanconi Anemia group A and C genes are viable, but display more severe sensitivity to DNA crosslinkers than either mutant alone. *M. Noll[1], K. Battaile[1], R. Bateman[1], C. Reifsteck[1], S.B. Olson[1], Y.M.N. Akkari[1], K. Rathbun[2], G. Bagby[2], A. D'Andrea[3], M. Grompe[1].* 1) Dept Molec/Medical Gen, L103, Oregon Health Sci Univ, Portland, OR; 2) VA Hospital, Portland, OR; 3) Harvard Medical Institute, Boston, MA.

Fanconi's Anemia (FA) is an autosomal recessive disorder characterized by birth defects, increased incidence of malignancy and progressive bone marrow failure. At least 8 complementation groups exist (FANCA-H) and 4/8 FA genes have been cloned. Here, we report the creation of a FANCA knock-out mouse, with a deletion of exon 37 resulting in a frame-shift and absent protein. FANCA is the most common defect in human FA, representing approximately 65% of all patients. Similar to FANCC mutants FANCA mutant mice were viable and had no gross developmental defects, but showed mild germ cell loss and hypersensitivity to DNA cross-linking agents. No hematologic abnormalities or cancer were observed. We next generated animals deficient in both FANCA and FANCC. Double mutants were born at the expected frequency and no macroscopic developmental abnormalities of the limbs or other organs were detected. Next, we established primary ear fibroblast cultures from FANCA, -C and A/C double mutants and control littermates. Treatment with MMC and DEB mutant cells revealed increased chromosome breakage in A/C double mutants compared to either mutant alone. This was corroborated by cell cycle analysis of primary cultures of MEFs exposed to 8-methoxypsoralen. Double mutant fibroblasts were at least five-fold more sensitive to the cross-linkers than the single mutants. Furthermore, histological analysis of both testis and ovaries showed a more profound germ cell loss in double mutants. Testicular weight was significantly reduced in the double mutants (101 mg) as compared to either FANCA -/- (158 mg), FANCC -/- mice (177 mg) or controls (214mg). Even at 6 months of age, no hematologic abnormalities or tumors have been observed A/C double mutants. We thus conclude that the FANCA and C proteins are at least partially non-epistatic and that mutation of either gene alone, does not render the FA dependent crosslinker response completely inactive.

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***ATM* mutation detection in lymphomas using oligonucleotide microarrays.** *J.G. Hacia[1], N.Y. Fang[1], T.C. Greiner[2], J.O. Armitage[2], W.C. Chan[2], J. Vose[2], D. Weisenburger[2], R.A. Mayer[2], F.S. Collins[1].* 1) GMMB, NHGRI/NIH, Bethesda, MD; 2) UNMC, Omaha, NE.

Ataxia telangiectasia (A-T) is an autosomal recessive disorder characterized by neurological degeneration, immune deficiency, and cancer predisposition. A-T patients demonstrate a 250-fold excess of lymphomas relative to the general population. To investigate the role *ATM* plays in the development and progression of sporadic lymphomas, we are analyzing over 120 DNA samples from follicular, mantle cell, diffuse large cell, peripheral T-cell, and post-transplant lymphomas for *ATM* mutations. We aim to identify subtypes commonly carrying ATM mutations and correlate this data with disease prognosis and responsiveness to treatment. Given the large size of the 9.4-Kb *ATM* coding region and its complex mutational spectrum, it is a challenge to rapidly and inexpensively scan for all possible sequence changes. We developed oligonucleotide microarray (DNA chip) assays to accomplish this task. *ATM* coding exons from patients and unaffected controls were added to DNA chips consisting of over 250,000 different probes. Mutations are detected through increased or decreased patient DNA hybridization to the arrayed probes relative to controls. Mutated exons identified by the DNA chip assays are sequenced to confirm the nature of the sequence change.

Over 90 lymphoma DNA samples have been screened for *ATM* mutations. A total of 5/25 mantle cell lymphoma samples showed deleterious mutations. These included three nonsense mutations and an in-frame deletion that removes two conserved amino acids. Two samples had missense mutations in conserved amino acids. No increased incidence of clearly deleterious mutations have been found in the other lymphoma subtypes.

We have successfully demonstrated the application of DNA chips to large-scale *ATM* mutation detection in tumor samples. *ATM* mutations may play a role in the development or progression of mantle cell lymphoma. We will evaluate germ-line mutational status to determine if carriers have an increased risk of developing certain lymphoma subtypes.